The use of transvaginal elastography diagnosing and dating of adenomyosis and in guiding the choice of treatment modality

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Imaging diagnosis of adenomyosis

- Transvaginal ultrasonography (TVUS)
  - Sensitivity: ~72%
  - Specificity: ~85%
  - AUC: 0.85

- MRI
  - Sensitivity: ~77%
  - Specificity: 89%
  - AUC: 0.93
Imaging diagnosis of adenomyosis

- Transvaginal ultrasonography (TVUS)
  - Sensitivity: ~72%
  - Specificity: ~85%
  - AUC: 0.85
- MRI
  - Sensitivity: ~77%
  - Specificity: 89%
  - AUC: 0.93
- "the diagnosis of adenomyosis remains a major challenge..." (Bazot & Darai 2018)
Improving the diagnostic accuracy by imaging techniques

• Standardization
  – MUSA
• Further training
• ...

Improving the diagnostic accuracy by imaging techniques

• Standardization (MUSA)
• Further training
• ...

• Have we reached the physical limit for TVUS and MRI?
Question

• Can better understanding of the pathogenesis/pathophysiology and the use of new emerging imaging technique help?
Pathogenesis vs. pathophysiology

• Pathogenesis:
  – What causes the disease?
  – Why is there a link between focal adenomyosis with DIE?

• Pathophysiology:
  – How does it cause pain/HUB?
  – Why is there enlarged uterus?
  – Why it seemingly defies medical treatment?
  – Why is there hyperinnervation in symptomatic patients?
  – Why is there hyper-/dys-peristalsis?

• From an interventional perspective, knowing pathophysiology is more important

• The most consequential:
  – Natural history
One defining hallmark of ectopic endometrium:

**Cyclic bleeding**
Bleeding/hemorrhage

- Indicative of vascular injury
- A quintessential hallmark of a wound or tissue damage
- Tissue repair is of paramount importance to the survival of an organism
- A defining feature shared by both endometriosis and adenomyosis
Key processes in the natural history of ectopic endometrium

Endometriotic stromal cells

Cyclic bleeding

Endometriotic epithelial cells

Smooth muscle cells

Desmin↑

SM-MHC↑

OTR↑

Platelet

TGF-β1

EMT

FMT

TGF-β1

E-cadherin↓

Vimentin↑

Snail, Slug↑

PAI-1↑

Invasiveness↑

TGF-β1

α-SMA↑

CTGF, LOX↑

Contractility↑

Collagen production↑


How about adenomyosis?
A serial experiment

Neonatal feeding of tamoxifen

Sampling of lesion tissues

Birth | D5 | D10 | D15 | D42 | D60

Parrott et al. 2001 AJP
Mehasseb and Habiba 2010 F&S
A: Depth of myometrial infiltration

\[ p = 1.1 \times 10^{-5} \]

Shen et al. HR 2016
B: Kinetics of hotplate latency

Day at hotplate test (since birth)

Latency (seconds)

Control

Adenomyosis

***

*** ***

#

NS

### #
A: Kinetics of VEGF staining

B: Kinetics of MVD

D: Kinetics of platelet aggregation

C: Kinetics of PCNA staining
E: Kinetics of TGF-beta1 staining

F: Kinetics of p-Smad3 staining

G: Kinetics of E-cadherin staining

H: Kinetics of vimentin staining

Shen et al. HR 2016
A: Kinetics of alpha-SMA staining

B: Kinetics of collagen I staining

C: Kinetics of LOX staining

D: Kinetics of PR-B staining

Shen et al. HR 2016
<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>D5</th>
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<th>D42</th>
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<td>SM-MHC</td>
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Histochemistry analysis

<table>
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<tr>
<th>Masson Trichrome</th>
<th>CTL</th>
<th>TAM</th>
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<td>D5</td>
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<tbody>
<tr>
<td>Staining</td>
<td>D5</td>
<td>D10</td>
</tr>
</tbody>
</table>

Shen et al. HR 2016
Pico-Sirius red staining of adenomyotic lesions under normal (a) and polarized microscopy (b-d).
Summary

• Our data are consistent with
  – Increased platelet aggregation
  – Increased activation of TGF-β/Smad3 signaling pathway
  – Progressive EMT, FMT, and SMM
  – Increased production of extracellular matrix
  – Increased fibrosis
  – Reduced hormonal receptor PR-B expression
    • Responsible for increased progestin-resistance?
## Validation in human adenomyosis

<table>
<thead>
<tr>
<th>Variable name</th>
<th>Controls (n=20)</th>
<th>Adenomyosis (n=34)</th>
<th>P-value</th>
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<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td>Mean=38.5 (SD=7.3)</td>
<td>Mean=44.6 (SD=5.4)</td>
<td>0.003</td>
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<tr>
<td></td>
<td>Median=40.5 range=25--48</td>
<td>median=45.0 Range=29--52</td>
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<tr>
<td><strong>Menstrual phase</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 (50.0%)</td>
<td>12 (35.3%)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>10 (50.0%)</td>
<td>22 (64.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5 (25.0%)</td>
<td>4 (11.8%)</td>
<td>0.47</td>
</tr>
<tr>
<td>1</td>
<td>12 (60.0%)</td>
<td>22 (64.7%)</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>3 (15.0%)</td>
<td>8 (23.5%)</td>
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<tr>
<td><strong>Visual analog scale on the severity of dysmenorrhea</strong></td>
<td>Mean=0.5 (SD=1.3)</td>
<td>Mean=436 (SD=2.9)</td>
<td>1.4x10^-5</td>
</tr>
<tr>
<td></td>
<td>Median=0.0 range=0--5</td>
<td>median=5.0 Range=0--8</td>
<td></td>
</tr>
<tr>
<td><strong>Uterus size (cm³)</strong></td>
<td>Median=70.4 Range=31.5—113.9 Missing: n=1</td>
<td>Median=229.6 Range=78.5—618.2</td>
<td>3.0x10^-8</td>
</tr>
<tr>
<td><strong>Co-occurrence of Endometriosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 (100.0%)</td>
<td>25 (73.5%)</td>
<td>0.019</td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0.0%)</td>
<td>9 (26.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Co-occurrence of Uterine fibroids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19 (95.0%)</td>
<td>33 (97.1%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (5.0%)</td>
<td>1 (2.9%)</td>
<td></td>
</tr>
</tbody>
</table>
CD42b: Marker for platelets
Vimentin: stromal cells
DAPI: nuclei

Liu et al. HR 2016
Density of CD42b+ platelets

Extent of platelet aggregation

Density

Control

Adenomyosis

p=0.017

Liu et al. HR 2016
Representative IHC results

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Adenomyosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-beta1</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>p-Smad3</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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<tr>
<td>E-cadherin</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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<tr>
<td>Vimentin</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
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<tr>
<td>alpha-SMA</td>
<td><img src="image9.png" alt="Image" /></td>
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<td>Collagen I</td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
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<tr>
<td>PR-B</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Liu et al. HR 2016
I: alpha-SMA

Control

Adenomyosis

Staining level

Group

p = 1.7 \times 10^{-9}

J: Collagen I

Control

Adenomyosis

Staining level

Group

p = 8.7 \times 10^{-13}

K: LOX

Control

Adenomyosis

Staining level

Group

p = 1.0 \times 10^{-7}

L: PR-B

Control

Adenomyosis

Staining level

Group

p = 5.5 \times 10^{-10}

Liu et al. HR 2016
A

Masson Trichrome Staining

Van Gieson Staining

B

Picro-sirius Red Staining
(Optical)

Picro-sirius Red Staining
(Polarized)
Based on Masson trichrome staining

Liu et al. HR 2016
M: Desmin

N: SM-MHC

O: OTR

Control Adenomyosis

Staining level

***

Group

Control Adenomyosis

Staining level

***

Group

Control Adenomyosis

Staining level

***

Group
The natural history of ectopic endometrium

Adenomyotic lesion

Platelets

Immune cells

Sensory nerves

EMT    FMT    SMM

Fibrogenesis

Enlarged uterus
Increased contractility
Hyperinnervation
Increased fibrosis
More pain
Epigenetic changes

Adenomyotic lesion

Fibrosis
Reduced vascularity

Time
Endometriosis is 'A fibrotic condition in which endometrial stroma and epithelium can be identified'

Time to redefine endometriosis including its pro-fibrotic nature

P. Vigano¹,*, M. Candiani², A. Monno³, E. Giacomini¹, P. Vercellini⁴, and E. Somigliana⁴
Adenomyosis is a condition started with the deposition of endometrial stroma and epithelium within the myometrium, which undergo cyclic bleeding and thus repeated tissue injury and repair, resulting in gradual and progressive smooth muscle metaplasia and fibrogenesis.
Fibrogenesis: An elephant in the room
The blind men and the elephant
Three basic requirements for a good theory

• It has to be falsifiable
  – Can be tested by experiments
• It can explain all existing data
• It can make useful predictions
Predictions

• Higher lesional fibrotic content should correlate with
  – More thorough EMT, FMT, and SMM
  – Higher tissue stiffness (by elastography)
  – Uterine size
  – More severe symptoms
  – Less progesterone responsiveness
  – Less vascularity
What is ultrasonic elastography?

- An ultrasound technique that measures the stiffness of tissues.

Palpation:
assessing the tenderness
of tissue through tissue
deforation

palpation imposes mechanical stress: shearing force & compression

Neil Roberts 2017
Different types of elastography

- **Strain imaging** generates images of relative tissue deformation

- **Shear wave imaging** induces and monitors shear wave propagation in tissue and reports a quantitative value related to the stiffness
Real-time ultrasonic elastography

An imaging technique to detect tissue strain while compressing surface with a transducer

Transducer (probe)
(Compress)
Surface
Ultrasound
Tumor, adenomyotic lesion

False color display of tissue strain
Soft tissue -> Large strain
Hard tissue -> Small strain

Strain = \frac{\text{del}(t)}{(T)}
HITACHI ALOKA
ARIETTA 70

Endovignal Probe:
EUP-C41V1(2-9MHZ)
Transvaginal elastosonography for diagnosing adenomyosis

Normal uterus (myo)  Uterine fibroids  Adenomyosis

By ReTIAR theory, we can diagnose and even date adenomyotic lesions

Liu et al. Reprod Sci 2018
Uterus size: 39x59x57 mm

Strain Ratio
- 1A: 0.19%
- 2B: 0.41%
- B/A: 2.14

Strain Histo
- MEAN: 90.27
- SD: 57.44
- %AREA: 37.18%
- COMP: 24.18
- KURT: 2.694
- SKEW: 0.58482
- CONT: 65.82
- ENT: 3.539
- IDM: 0.2205
- ASM: 0.000497
- CORR: 0.9899
- LF Index: 2.585
Elastography applied to patients with adenomyosis and uterine fibroids

• Two data sets
  – Set 1: Adenomyosis (n=35), uterine fibroids (n=8), control uteri (CIN III, n=11)
    • Elastography
    • Hysterectomy
    • IHC analysis for markers of EMT, FMT, and fibrosis, ER and PR
  – Set 2: AM (n=112), UF (n=67), Control (n=130)
    • Elastography
    • Some went hysterectomy
Transvaginal elastosonography for diagnosing adenomyosis

A: Tissue stiffness among different groups

B: Lesional stiffness between different types of adenomyosis

C: Lesional stiffness vs. uterine size in adenomyosis

D: Tissue stiffness vs. uterine size

Liu et al. Reprod Sci 2018
<table>
<thead>
<tr>
<th>E-cadherin</th>
<th>Eutopic EM</th>
<th>Ectopic EM (low stiffness)</th>
<th>Ectopic EM (high stiffness)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>α-SMA</th>
<th>Eutopic EM</th>
<th>Ectopic EM (low stiffness)</th>
<th>Ectopic EM (high stiffness)</th>
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</table>

<table>
<thead>
<tr>
<th>ER-β</th>
<th>Eutopic EM</th>
<th>Ectopic EM (low stiffness)</th>
<th>Ectopic EM (high stiffness)</th>
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</table>

<table>
<thead>
<tr>
<th>PR</th>
<th>Eutopic EM</th>
<th>Ectopic EM (low stiffness)</th>
<th>Ectopic EM (high stiffness)</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Control Myometrium</th>
<th>Uterine Fibroid</th>
<th>ADM (low stiffness)</th>
<th>ADM (high stiffness)</th>
</tr>
</thead>
</table>

(a) Control Myometrium (b) Uterine Fibroid (c) ADM (low stiffness) (d) ADM (high stiffness)
A: Extent of lesional fibrosis vs. lesional stiffness

B: E-cadherin staining vs. lesional stiffness

C: alpha-SMA staining vs. lesional stiffness

D: ER-beta staining vs. lesional stiffness

E: PR staining vs. lesional stiffness

F: Extent of fibrosis/PR staining ratio vs. lesional stiffness
Conclusions

• In light of the natural history of adenomyosis, transvaginal ultrasonic elastography can be used to diagnose adenomyosis

• Adenomyosis can be diagnosed and staged by elastography

• Elastography is superior to conventional ultrasound in differential diagnosis

• Elastographic finding may also be used to guide the choice of the best treatment modality
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7th Asian Conference on Endometriosis

September 14-16, 2018
Taipei, Taiwan

www.acetaiwan2018.org/